THE CYTOLOGY OF SOME CULTIVATED SPECIES OF VIBURNUM

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Viburnum has long been recognized as a difficult group taxonomically, and more recently it has been shown to be somewhat complex cytologically. In recent years evidence from cytological studies has been used extensively in formulating taxonomic conclusions, but all too often only a small percentage of the species in a genus is studied cytologically, and, on the basis of these, generalizations are made concerning the entire genus. This has led to erroneous conclusions, both in cytology and taxonomy. It is hoped that additional studies in *Viburnum* will lead to a better understanding of the cytology of the genus, and that this, in turn, can be used in developing a sound and workable taxonomy.

The first chromosome counts in *Viburnum* were published in 1930 by Sax and Kribs (5) in a study of the cytology and wood structure in the Caprifoliaceae. In this paper chromosome numbers were reported for eleven species of *Viburnum* representing six of the nine sections of the genus, according to the classification of Rehder (3), and including both Asiatic and American species. All of the species included in this study were found to have nine pairs of chromosomes. However, in 1932, Simonet and Miedzyrzecki (6) reported that *Viburnum fragrans* has only eight pairs of chromosomes and, in 1936, Sugiura (7) reported that *V. awabuki* (*V. odoratissima*) has ten pairs of chromosomes. The most comprehensive study of the chromosomes of *Viburnum* was that of Janaki Ammal (1) in which a survey was made of the species growing in the botanical gardens at Wisley, Kew, and Paris. In this paper, chromosome counts were reported for the first time for twenty-three species and varieties, and additional counts were given for sixteen species for which counts had been reported previously by other authors. The present study was undertaken to extend the survey of chromosome numbers in *Viburnum* and to see if further evidence could be obtained on the origin of the aneuploid series in the genus. The chromosome counts obtained in this study, along with all other counts for *Viburnum* which I have seen, are listed in Table I. The classification used in this table is based on that of Rehder (4).

MATERIALS AND METHODS

The plant materials used in this study were from the living collection on the grounds of the Arnold Arboretum. Most of the chromosome counts were made from acetocarmine squashes of pollen mother cells. Satisfactory preparations were obtained by squashing fresh, whole anothers on a dry

TABLE I. **Chromosome Counts in Viburnum.**

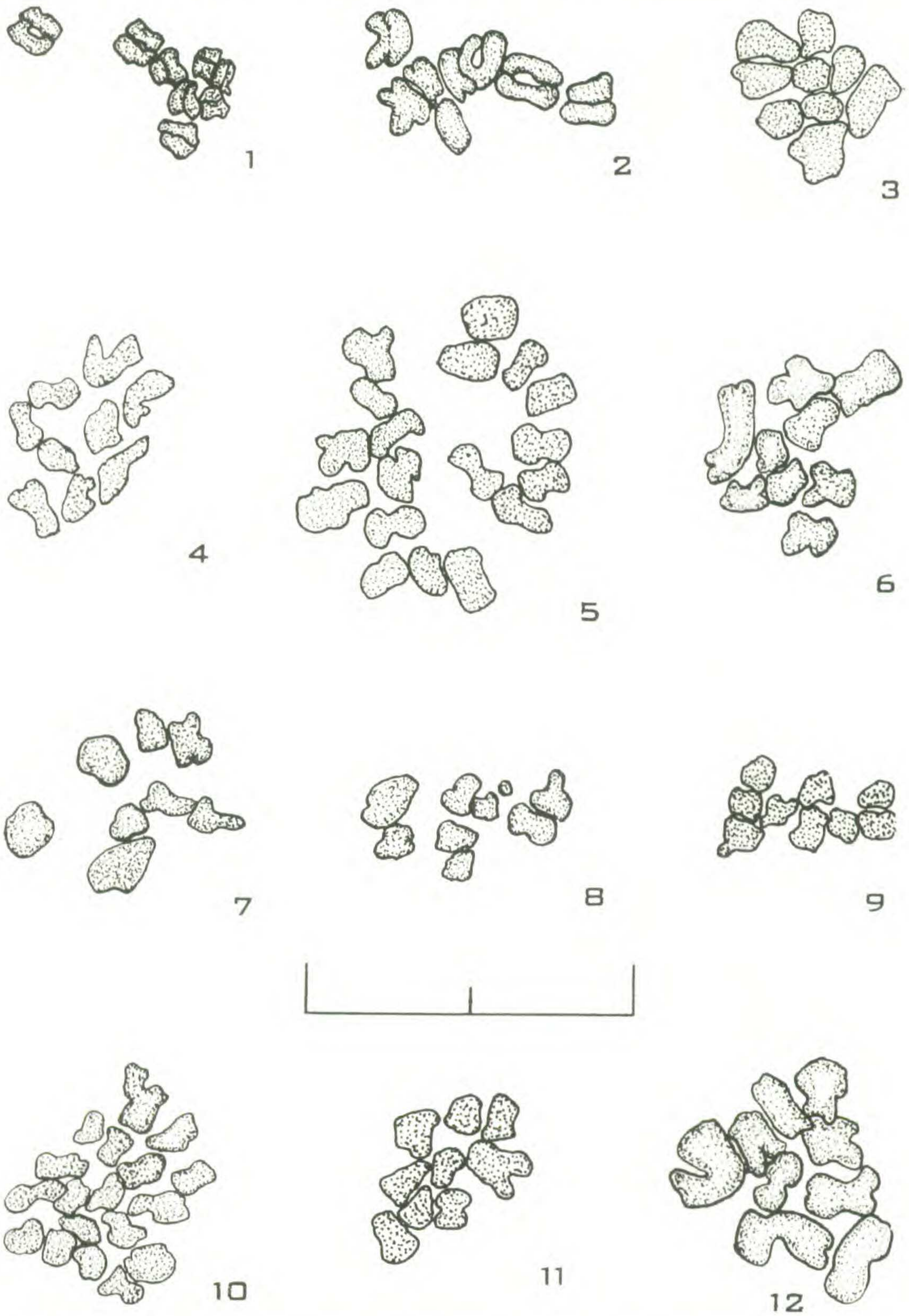
SPECIES	2n	AUTHORITY *	DOCUMENTATION †	DISTRIBUTION
Sect. THYRSOSMA				
V. erubescens Wall.	48	1	none	Himal., w. China
V. foetens Decne.	16	1	none	Himal., Kashmir
V. fragrans Bunge	16	1, 6	none	n. China
var. *candidissima*	16	8	55–50–B	cult.
var. *alba*	32	1	none	n. China
V. grandiflorum Wall.	16	1	none	Himal.
V. henryi Hemsl.	48	1	none	centr. China
V. odoratissimum Ker-Gawl.	40	1	none	India, China
V. seiboldii Miq.	16	1	none	Japan
"	32	8	AA616–6–B	"
V. suspensum Lindl.	16	1	none	Liukiu Islands
Sect. LANTANA				
V. bitchiuense Makino	16	1	none	w. Japan
V. buddleifolium Wright	20	1	none	centr. China
"	18	6	none	"
V. × *burkwoodii* Burkwood & Skipwith (*V. carlesii* × *utile*)	18	8	AA815–41–B	cult.
V. × *carlcephalum* Burkwood ex. Pike (*V. carlesii* × *macrocephalum*)	18	8	AA618–53–A	cult.
V. carlesii Hemsl.	20	1	none	Korea
"	18	2, 6, 8	AA17981–A	"
V. cotinifolium D. Don	18	2	none	Himal.
V. lantana L.	18	1, 2, 5	none	Eur., w. Asia
var. *rugosum* Lange	18	8	AA907–27–A	cult.
V. mongolicum (Pall.) Rehd.	16	1	none	e. Siberia, n. China
V. × *rhytidophylloides* Suringar (*V. rhytidophyllum* × *lantana*)	18	8	AA711–36–B	cult.
V. rhytidophyllum Hemsl.	18	1, 6	none	centr. & w. China
V. utile Hemsl.	18	1	none	centr. China
V. urceolatum Seib. & Zucc.	18	8	AA876–51	Japan
V. veitchii Wright	18	8	AA7198	centr. China
Sect. PSEUDOTINUS				
V. alnifolium Marsh.	18	1, 5	none	e. N. Am.
V. furcatum Blume ex Maxim.	18	1	none	Japan
Sect. PSEUDOPULUS				
V. plicatum Thunb.	16	8	AA18016–1	Japan, China
f. *lanceatum* Rehd.	16	8	AA6122–1	cult.
f. *mariesii* (Veitch) Rehd.	18	8	AA870–51–A	cult.
f. *roseum* (Doney) Rehd.	16	8	AA856–34	cult.

TABLE I (*Continued*)

SPECIES	2*n*	AUTHORITY *	DOCUMENTATION †	DISTRIBUTION
Sect. LENTAGO				
V. cassinoides L.	18	8	AA17997	e. N. Am.
V. lentago L.	18	1, 5	none	e. N. Am.
V. nudum L.	18	1	none	e. N. Am.
V. prunifolium L.	18	1, 5	none	e. N. Am.
Sect. TINUS				
V. cinnamomifolium Rehd.	18	1	none	w. China
V. davidii Franch.	18	1	none	w. China
V. harryanum Rehd.	18	2	none	w. China
V. propinquum Hemsl.	18	2	none	centr. & w. China
V. tinus L.	36	1, 2, 6	none	se. Eur.
Sect. ODONTOTINUS				
V. acerifolium L.	18	1, 5	none	e. N. Am.
V. betulifolium Batalin	18	1	none	centr. & w. China
V. dentatum L.	54	1	none	e. N. Am.
"	36	8	AA17985	"
var. *pubescens* Ait.	36	8	AA18009	"
V. dilatatum Thunb.	18	8	AA229–46–B	e. Asia
f. *hispidum*	18	8	AA17486–1–A	cult.
f. *xanthocarpum* Rehd.	18	8	AA10140	cult.
V. foetidum Wall.	16	1	none	w. China
var. *rectangulatum* (Graebn.) Rehd.	16	1	none	w. China
V. hirtulum Rehd.	18	8	AA708–37–B	China
V. hupehense Rehd.	18	5, 6	none	China
V. lobophyllum Graebn.	20, 22	1	none	centr. & w. China
"	18	5	none	"
V. molle Michx.				
f. *leiophyllum* Rehd.	18	8	AA4643–1–A	e. N. Am.
V. ovatifolium Rehd.	18	1, 8	AA20078A	w. China
V. pubescens Pursh	36	8	AA18008	e. N. Am.
V. raffinesquianum Roem. & Schult. var. *affine* (Schneid.) House	20	8	AA4622–2–B	e. N. Am.
V. setigerum Hance	36	8	AA20189	centr. & w. China
" (as *V. theiferum* Rehd.)	18	6	none	"
f. *aurantiacum* Rehd.	36	8	AA812–32	"
V. wrightii Miq.	16	1	none	Japan
Sect. OPULUS				
V. opulus L.	18	1, 5	none	Eur. & n. Africa
f. *xanthocarpum* (Endl.) Rehd.	18	8	AA1298–28–A	cult.
V. sargentii Koehne	18	5	none	ne. Asia
f. *calvescens* (Rehd.) Rehd.	18	8	AA467–26	cult.
V. trilobum Marsh.	18	1, 5	none	n. N. America
'compactum'	18	8	AA871–51	cult.

* 1, Janaki Ammal; 2, Poucques; 5, Sax; 6, Simonet and Miedzyrzecki; 7, Sugiura; 8, Thomas, reported here for the first time.

† The numbers listed under this column are the accession numbers for the plants in the living collection of the Arnold Arboretum. Voucher specimens of these plants bearing the accession number have been deposited in the herbarium of the Arnold Arboretum.



Figs. 1–12. Chromosomes of Viburnum. 1, *V. fragrans* var. *candidissima*, metaphase II; 2, *V. carlesii*, late metaphase I; 3, *V. ovatifolium*, metaphase I; 4, *V. rhytidophylloides*, telophase II; 5, *V. pubescens*, metaphase I; 6, *V. veitchii*, metaphase I; 7, *V. plicatum*, metaphase I; 8, *V. plicatum* f. *mariesii*, metaphase I; 9, *V. dilatatum* f. *hispidum*, metaphase II; 10, *V. dentatum*, metaphase I; 11, *V. dilatatum* f. *xanthocarpum*, metaphase II; 12, *V. opulus* f. *xanthocarpum*, metaphase I. Each division in the scale represents 10 micra.

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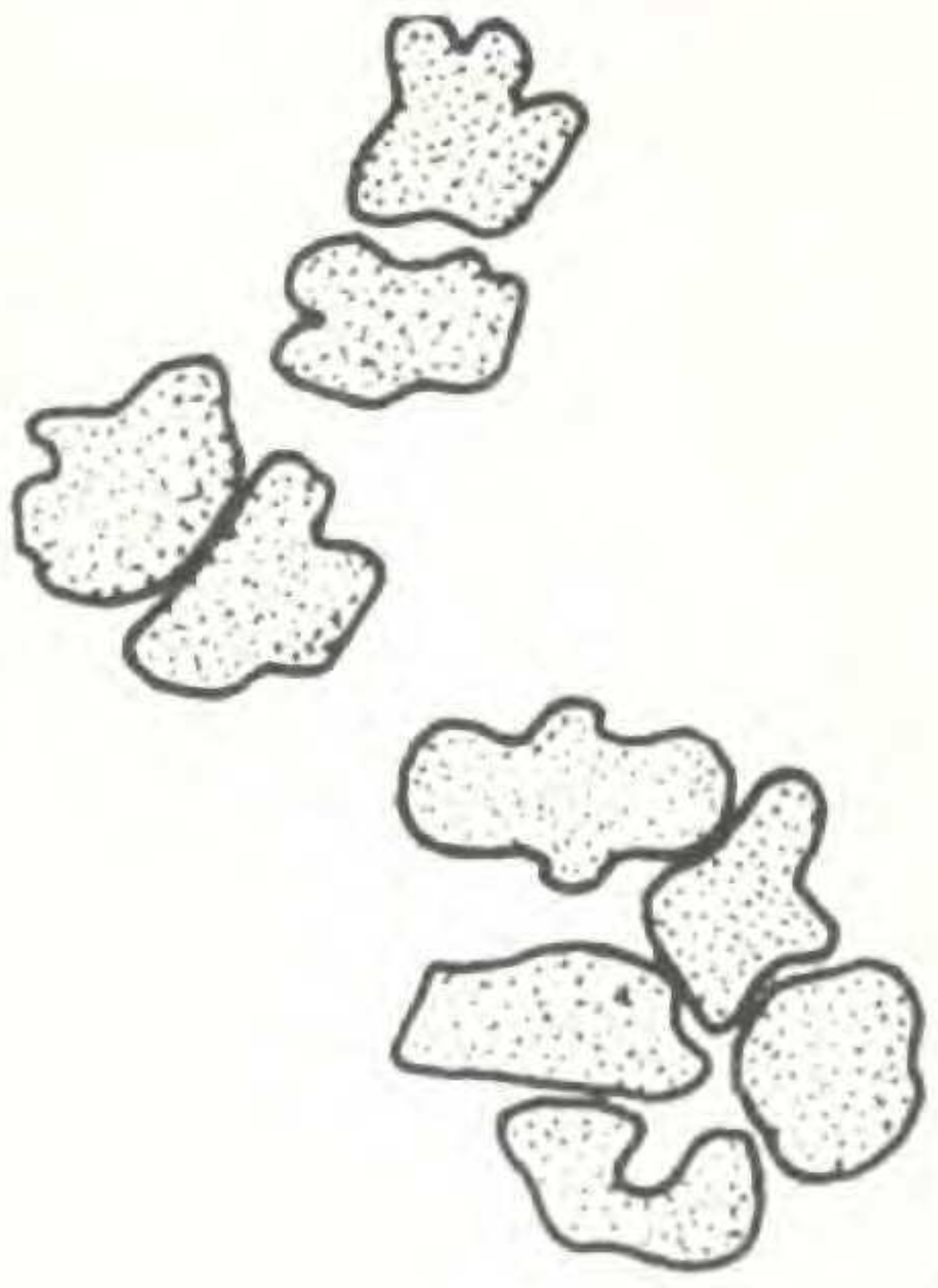

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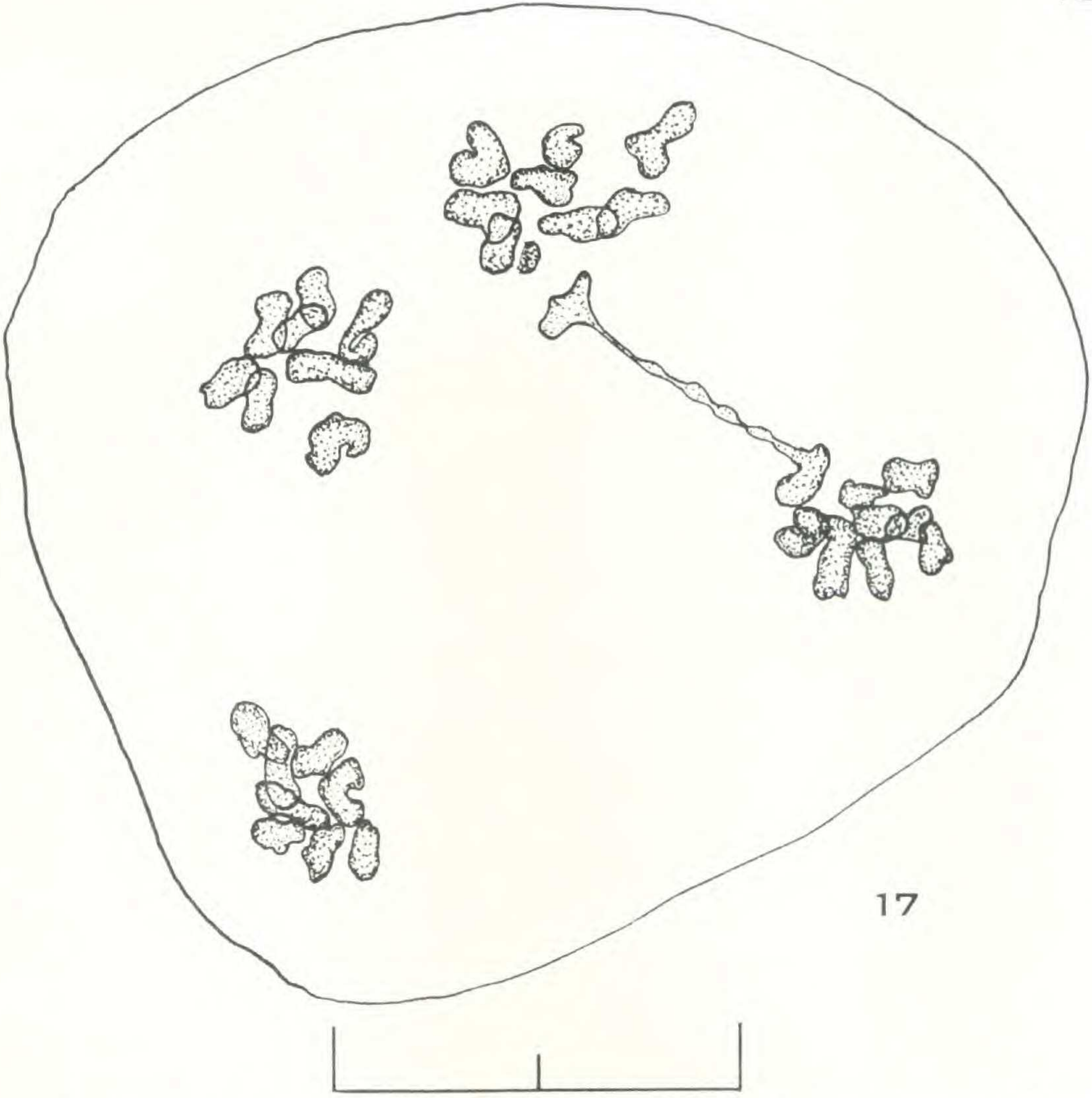

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FIGS. 13–17. CHROMOSOMES OF VIBURNUM. 13, *V. cassinoides*, metaphase I; 14, *V. urceolatum*, metaphase I; 15, *V. rafinesquianum* var. *affine*, metaphase I; 16, *V. lantana* var. *rugosum*, metaphase II; 17, *V. rhytidophylloides*, telophase II, showing loss of a chromosome due to unequal translocation. Each division in the scale represents 10 micra.

slide, immersing the slide in Carnoy's fixative for ten to twenty minutes, and staining the material with acetocarmine. In a few cases, counts were made on root tips obtained from rooted cuttings. The root tips were treated for two to three hours in 0.1% colchicine, fixed in Carnoy's solution, softened in concentrated HCl for ten to fifteen minutes, and stained with acetocarmine.

The time of flowering varies considerably in different species of *Viburnum*, and meiosis often occurs over a short period of time. In order to avoid missing meiosis in several species, small cuttings were brought indoors and forced. Most species forced readily after the middle of March and it was much easier to follow their development under the relatively uniform conditions in the laboratory, than under the highly variable spring weather conditions outside.

ORIGIN OF THE ANEUPLOID SERIES

Janaki Ammal (1) has postulated the origin of the chromosome numbers nine and ten starting with a base number of eight for the genus. The number ten was thought to have originated from a backcross between a chance triploid ($2n = 24$) and a diploid ($2n = 16$) to produce $2n = 20$. Plants with this number are referred to as "secondary diploids." The number nine was said to have originated from hybridization between plants having $2n = 20$ and $2n = 16$, and are referred to as "tertiary diploids." The origin of *Viburnum juddii* was cited as an example of the latter.

This explanation is very logical and it is possible that some species of *Viburnum* have originated in this manner. However, in view of the evidence at hand it seems more likely that species with chromosome numbers of eight and ten originated from species with a base number of nine by the gain or loss of a chromosome. The base number nine is by far the dominant number in the Caprifoliaceae, as well as in the genus *Viburnum*. Of the 50 species of *Viburnum* for which chromosome counts are available (not including varieties, forms, or cultivars) twelve have a base number of eight, 33 have nine, and only two have undisputed counts of $x = 10$. Three additional species were reported by some workers to have $x = 10$ but by other workers to have $x = 9$. It seems probable that the discrepancies here are due to real differences in the chromosome numbers of the plants counted, rather than to mistakes in counting. There are no discrepancies reported for species with $x = 8$, whereas three of the five species reported to have $x = 10$ also have reported counts of nine, and one of these also has a count of eleven. Thus, the number ten seems to be relatively unstable, which is what one would expect if it originated by the gain of a chromosome from species with nine. The duplicated chromosome material is apparently lost with little or no deleterious effect on the plant.

Among the species in which there is a discrepancy in the reported chromosome numbers *Viburnum carlesii* is particularly significant. As mentioned above, Janaki Ammal used the case of *V. juddii* to illustrate the origin of the nine-chromosome series. *Viburnum juddii* is an openpollinated offspring of *V. carlesii*, with *V. bitchiuense* suggested as the most probable male parent. The chromosome counts reported for these plants by the above author were as follows: *V. carlesii*, $2n = 20$; *V. bitchiuense*, $2n = 16$; and *V. juddii*, $2n = 18$. However, Poucques (2) and Simonet and Miedzyrzecki (6) reported counts of $2n = 18$ for *V. carlesii*, and, upon checking the original plant from which *V. juddii* originated, I found that it, too, has $2n = 18$. It is fortunate, indeed, that the original plant is still growing on the grounds of the Arboretum and that thorough records were kept documenting the female parent of this hybrid species. I have not yet examined the plants of *Viburnum bitchiuense* which are growing in the vicinity of *V. carlesii*, but if they do have a chromosome number of $2n = 16$, as reported, it seems highly unlikely that this species served as the male parent of *V. juddii*.

Chromosome aberrations were fairly common in several of the species of *Viburnum* which I examined, and unequal divisions were noted in several instances. Translocations apparently occurred rather frequently as evidenced by bridge formations observed in several of the species (see FIG. 17). A relatively high percentage of aberrations was noted in *Viburnum cassinoides*, *V. carlcephalum*, *V. carlesii*, *V. dentatum*, *V. plicatum*, *V. rhytidophylloides*, *V. seiboldii*, *V. trilobum* f. *compactum*, and *V. veitchii*.

In a situation such as this the opportunities for gaining or losing a chromosome are greatly increased, and with frequent translocations the chances of losing a chromosome and still retaining a viable chromosome complement are also increased. An example of the gain of a chromosome is that of *Viburnum plicatum* f. *mariesii*, which originated in cultivation. *Viburnum plicatum* and two of its cultivated forms, f. *lanceatum* and f. *roseum* have $2n = 16$, whereas f. *mariesii* has an extra pair of chromosomes which are very small but nevertheless apparently pair and divide normally during meiosis.

Viburnum is still in need of further cytological work to give a more complete picture of the natural distribution of the three chromosome series. Less than half of the species have been studied cytologically, these being cultivated species, for the most part, and, in many cases, species that have been in cultivation for a long time. Additional cytological work, well documented with herbarium specimens, is needed particularly on Asiatic species from the wild.

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